PB88-125455

Evaluation of the Genetic Activity Profiles of 65 Pesticides

Environmental Health Research and Testing, Inc.
Research Triangle Park, NC

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1986

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RE PB88-125455
EPA/600/J-86/415		

4. TITLE AND SUBTITLE (Journal Version)

Evaluation of Genetic Activity Profiles for Sixty-five Pesticides

5. REPORT DATE: 1986

6. PERFORMING ORGANIZATION CODE:

7. AUTHOR(S) Neil E. Garrett[1], H. Frank Stack[1], and Michael D. Waters[2] [1]EHRAT, Inc., P.O. Box 12199 RTP, NC 27709, and [2]U.S.EPA, MD-68, RTP, NC 27711

9. PERFORMING ORGANIZATION NAME AND ADDRESS
[1]Environmental Health Research and Testing, Inc., P.O. Box 12199, Research Triangle Park, NC 27709; [2]Genetic Toxicology Division, Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC 27711

8. PERFORMING ORGANIZATION REPORT NO:

10. PROGRAM ELEMENT NO.: CCAE1A

11. CONTRACT/GRANT NO.: 68-02-4031

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED:

14. SPONSORING AGENCY CODE: EPA-600/11

15. SUPPLEMENTARY NOTES
Mutation Research, 168(1986) 301-325

16. ABSTRACT

We have previously reported the qualitative results of a major study on 65 pesticides (Waters et al., 1982). Dose information from this investigation (either lowest effective or highest ineffective dose tested) has now been incorporated into a computerized data management system. This report focuses on the quantitative profiles of genetic activity produced by these pesticides and our efforts to classify them according to their genotoxic effects and chemical structures. Three main categories may be distinguished based on the qualitative results: Category 1 pesticides were active in most of the in vitro and in vivo assays employed. These nine compounds include the structurally similar organophosphate insecticides, acephate, dazoton, monocrotophos, and trichlorfon; the phthalimid fungicide analogues, captan and folpet; and the thiocarbamate herbicide analogues, diallate, sulfallate and triallate. The 26 Category 2 compounds demonstrated fewer positive results and may be subdivided into two parts, one of which contains 12 halogenated aromatic or heterocyclic ring compounds, including the phenoxy herbicides, 2,4-D, 2,4-DB, and 2,4,5-T. The remaining part of Category 2 (14 compounds) consists of structurally similar organophosphate insecticides, azinphos-methyl, crotoxyphos, disulfoton, methyl parathion; three similar ethylenebisdithiocarbamate fungicides, maneb, mancozeb, and zineb; three similar pyrethroid insecticides, allethrin, chrysanthemic acid, and ethy chrysanthemate; and four structurally diverse compounds, cacodylic acid, dinoseb, sec-butylamine and benomyl. The third category of 30 pesticides gave negative results in all tests and represents structurally diverse compounds. Using the computerized profile matching methodology, from 2080 possible pairwise chemical combinations of the 65 pesticides, 20 statistically significant pairs were selected. Six groups of pesticides were identified which were substantially similar to groups of pesticides we had formed previously (Waters et al., 1982) based on genetic activity and chemical structure. The matches showed excellent qualitative and, in most cases, excellent quantitative agreement. Hence it appears that specific patterns of test results present in the genetic activity profiles are related directly to chemical structure. Conversely, the data suggests that certain groups of compounds may be recognized by a well defined series of concordant tests results. As additional data is added, comparison of test results for new chemicals with existing data for known genotoxicants should aid in the evaluation of potential genetic health hazards.

(Copyright (c) 1986 Elsevier Science Publication B.V.)

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field. Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	27
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE PC 12.95 / MF 6.95

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA/600/J-86/415
JOURNAL ARTICLE

Mutation Research, 168 (1986) 301–325
Elsevier

MTR 07207

Evaluation of the genetic activity profiles of 65 pesticides

Neil E. Garrett [1], H. Frank Stack [1] and Michael D. Waters [2,*]

[1] *Environmental Health Research and Testing, Inc., P.O. Box 12199, Research Triangle Park, NC 27709 (U.S.A.);*
[2] *Genetic Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 (U.S.A.)*

(Received 16 April 1986)
(Accepted 21 April 1986)

Summary

We have previously reported the qualitative results of a major study on 65 pesticides (Waters et al., 1982). Dose information from this investigation (either lowest effective or highest ineffective dose tested) has now been incorporated into a computerized data management system. This report focuses on the qualitative profiles of genetic activity produced by these pesticides and our efforts to classify them according to their genotoxic effects and chemical structures. Three main categories may be distinguished based on the qualitative results: Category 1 pesticides were active in most of the in vitro and in vivo assays employed. These 9 compounds include the structurally similar organophosphate insecticides, acephate, demeton, monocrotophos and trichlorfon; the phthalimide fungicide analogues, captan and folpet; and the thiocarbamate herbicide analogues, diallate, sulfallate and triallate. The 26 Category 2 compounds demonstrated fewer positive results and may be subdivided into two parts, one of which contains 12 halogenated aromatic or heterocyclic ring compounds, including the phenoxy herbicides, 2,4-D, 2,4-DB and 2,4,5-T. The remaining part of Category 2 (14 compounds) consists of structurally similar organophosphate insecticides, azinphos-methyl, crotoxyphos, disulfoton, methyl parathion; three similar ethylenebisdithiocarbamate fungicides, maneb, mancozeb, and zineb; three similar pyrethroid insecticides, allethrin, chrysanthemic acid, and ethyl chrysanthemate; and four structurally diverse compounds, cacodylic acid, dinoseb, *sec.*-butylamine and benomyl. The third category of 30 pesticides gave negative results in all tests and represents structurally diverse compounds. Using the computerized profile matching methodology, from 2080 possible pairwise chemical combinations of the 65 pesticides, 20 statistically significant pairs were selected. 6 groups of pesticides were identified which were substantially similar to groups of pesticides we had formed previously (Waters et al., 1982) based on genetic activity and chemical structure. The matches showed excellent qualitative and, in most cases, excellent quantitative agreement. Hence it appears that specific patterns of test results present in the genetic activity profiles are related directly to chemical structure. Conversely, the data suggests that certain groups of compounds may be recognized by a well defined series of concordant tests results. As additional data is added, comparison of test results for new chemicals with existing data for known genotoxicants should aid in the evaluation of potential genetic health hazards.

---

* Reprint requests should be directed to MDW.

The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

0165-1110/86/$03.50 © 1986 Elsevier Science Publishers B.V. (Biomedical Division)

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

The purpose of this paper is 2-fold: first, to examine short-term test data for 65 pesticide chemicals, and second to use these data as a model in further developing the concept and use of genetic activity spectra or profiles. These profiles provide a graphic representation of bioassay data that facilitates both visual and computer-assisted comparative assessment.

The concept of a genetic activity profile for a chemical originated from the need to represent in a single two-dimensional configuration the qualitative and quantitative data from a large number of genetic bioassay systems (currently more than 200). Individual bioassay systems measure point/gene mutation, chromosomal effects, DNA damage, cell transformation, and in some studies effects on sperm. The *x*-axis values of the profile represent the bioassays in an endpoint/phylogenetic sequence and the *y*-axis values represent the logarithmically-transformed lowest effective doses (LED) or the highest ineffective doses (HID) tested. In practice, the profile is constructed as a computer generated bar graph.

We have previously reported genetic activity profiles for 24 agents classified by the International Agency for Research on Cancer (IARC) as known or suspected human carcinogens (Garrett et al., 1984) and profiles for several well-known organic solvents (Vainio et al., 1985). IARC subsequently published genetic activity profiles for an additional group of 13 compounds including allyl compounds, aldehydes, epoxides and peroxides (IARC, 1985). The U.S. National Academy of Sciences recently published genetic activity profiles for cyclamate, cyclohexamine, and *N*-hydroxycyclohexylamine (NAS, 1985). In addition, chemicals that induce aneuploidy have been presented using the genetic activity profile method (Waters et al., 1985, 1986). To date, genetic activity profiles have not been reported for pesticide chemicals.

The impact of pesticides on the health of agricultural workers and consumers is still largely unknown. Genotoxic effects are considered among the most serious of the possible side effects of agricultural chemicals. Such effects are of special concern because of the generally irreversible nature of the processes and the long latency associated with their manifestation. Continued attention should be focused on pesticides applied to crops or soil because of the large amounts of these chemicals currently in use, the extent of field worker exposure and the possibility of direct entry of pesticides into the food chain.

We have reported previously aspects of the qualitative bioassay data for the 65 pesticides described in this report (Waters et al., 1982). Dose information has been incorporated into the pesticide data base (670 test results) and the profile methodology has been used to compare systematically the genetic activity of these agents.

Methods

Descriptions of each bioassay system, procurement of pesticides, purity and qualitative aspects of the pesticide data, were reported previously (Waters et al., 1982). Chemical structures were provided by STN International (American Chemical Society) from a CAS on-line data base. All bioassays except 29 of the Drosophila sex-linked recessive lethal tests were performed by SRI International, Menlo Park, CA. The Drosophila assays were performed by WARF Institute, Inc., Madison, WI. Table 1 lists the 14 bioassays employed as they are divided into classes of genetic damage according to phylogenetic level of the test organisms (prokaryotic or eukaryotic). Each bioassay is denoted by a three-character descriptor in accordance with the EPA Gene-Tox Program (Waters, 1979). 6 bioassays in the 'initial test battery' were applied to 51 of the 65 chemicals in the study. 500 bioassays have been performed including 300 in vitro tests with and without an exogenous metabolic system (Aroclor 1254 induced rat-liver S9).

Determination of effective doses

The term 'dose' in this report does not imply consideration of exposure or treatment time and, can be considered synonymous with concentration. Either the lowest effective dose or the highest dose tested that produced no effect was recorded for each test agent and bioassay system.

For each negative test result, the highest dose studied was termed the highest ineffective dose (HID). Similarly, for positive results, the lowest effective dose (LED) was recorded. Theoretically,

TABLE 1

GENOTOXICITY BIOASSAYS EMPLOYED

Descriptor	Organism	Property examined
Point / gene mutations in Prokaryotes		
SAL [a]	*S. typhimurium* (3 strains)	Reverse mutation
WPU [a]	*E. coli* WP2 *uvrA*	Reverse mutation
Point / gene mutations in Eukaryotes		
YER	*S. cerevisiae* D7	Reverse mutation
L5T	Mouse lymphoma L5178Y cells, TK locus	Forward mutation
SRL	*D. melanogaster*	Recessive lethality
Primary DNA damage in Prokaryotes		
REP [a]	*E. coli polA*	Differential toxicity
REW [a]	*B. subtilis rec*	Differential toxicity
SAR	*S. typhimurium uvrB, rec*	Differential toxicity
Primary DNA damage in Eukaryotes		
YE3 [a]	*S. cerevisiae* D3	Enhanced mitotic recombination
YEH	*S. cerevisiae* D7	Gene conversion and crossing-over
UDH [a]	Human lung fibroblasts WI-38	Unscheduled DNA synthesis
Chromosomal effects		
SCC	Chinese hamster ovary cells	Sister-chromatid exchange
MNM	Mouse bone marrow and cardiac blood	Chromosome breakage (micronuclei)
DLM	Mouse	Dominant lethality

[a] A bioassay from the initial battery of screening tests.

The bioassays SAL, WPU, YER, L5T, YE3, YEH, UDH and SCC were carried out both in the presence and absence of Aroclor-1254 induced rat-liver S9. SAL is represented in the genetic profiles by codes identifying specific tester strains (SA7 = TA1537, SA9 = TA98, and SA0 = TA100).

the HID and LED both approach the no-observed-effect level as their limit (Chan et al., 1982).

In practice, the doses or concentrations for all in vitro tests were converted to μg/ml and those for in vivo tests were expressed as mg/kg. Because of the wide range of doses encountered in the bioassays and to accommodate both positive and negative responses on a continuous scale, doses were transformed logarithmically so that effective (LED) and ineffective (HID) doses were represented by positive and negative numbers, respectively. The responses or logarithmic dose unit (LDU_{ij}) for a given test system i and chemical j was represented by the expressions

$$LDU_{ij} = -\log_{10}(\text{dose}), \text{ for HID values; } LDU \leq 0$$

$$LDU_{ij} = -\log_{10}(\text{dose} \times 10^{-5}), \text{ for LED values; } LDU \geq 0 \quad (1)$$

These simple relationships define a dose range of 0 to −5 logarithmic units for ineffective doses (1–100000 μg/ml or mg/kg) and 0 to +5 logarithmic units for effective doses (100000–1 μg/ml or mg/kg). A scale illustrating the LDU values is shown in Fig. 1. Negative responses at doses less than 1 were set equal to 1. Effectively, an LED value ≥ 100000 or an HID value ≤ 1 produces an $LDU = 0$; therefore, no quantitative information is gained from such extreme values.

Because dose units were plotted on a log scale, differences in molecular weights of compounds did not greatly influence comparisons of their overall genetic activity profiles.

Representation of a genetic activity profile

The genetic activity profile for each chemical in the data base was represented by the different test systems on the x-axis and by values corresponding to the LDU for each individual test system on the

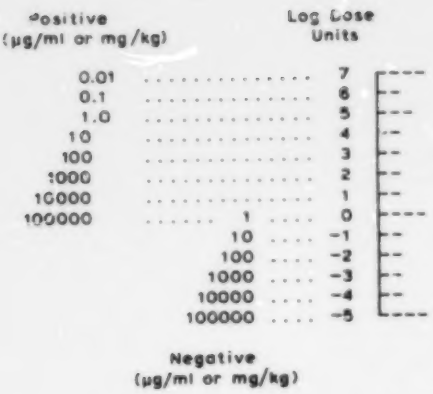


Fig. 1. Scale corresponding to the *y*-axis used for the genetic activity profiles.

y-axis. The bioassays are grouped according to classes of genetic or genetic-related activity and arranged within the classes according to phylogenetic level. Thus, a discrete array of tests and responses defines a readily interpretable profile or spectrum of genetic activity.

Spectral-line height (the magnitude of each bar) is a function of the LED or HID, which is associated with characteristics of each individual test system, such as population size, cell-cycle kinetics and metabolic competency. Thus, the detection limit of each test system is different, and across a given profile responses will vary substantially. The profiles were compared and analyzed only on the basis of common test results (i.e., where the same test systems were used to evaluate two or more of the chemicals under consideration). No effort was made to adjust or relate responses in one test system to those of another.

Data management and comparative computer analysis

To determine patterns or similarities in test data, the data were examined over the entire series of tests for all common tests conducted for each pair of chemicals. The type and number of common tests and the number of common tests with conflicting results were computed. The significant chemical combinations were determined by calculating the probability (P) that the number of concordant test results would occur by chance. This is given by the relation:

$$P = \sum_{i=n-m}^{n} \binom{n}{i} (0.5)^n \qquad (2)$$

where 0.5 is the probability of the results matching for any common tests, n is the number of common tests, m is the number of conflicting tests, and $\binom{n}{i}$ are the binomial coefficients defined as $n!/[(n-i)!i!]$, $n \geq i \geq 0$.

The matching process for the pesticides was performed in two stages. The first stage considered all matches of $P \leq 0.05$. The second stage considered only matched at $P \leq 0.02$.

Additionally, the dose-related features of the genetic profile for common tests of the pesticides were compared. This comparison was made by first calculating the difference in the values of the logarithmic responses,

$$| LDU_{ij_a} - LDU_{ij_b} |$$

where j_a and j_b denote the two chemicals being compared, and i the test system. A dose-related function (DRF) has been defined to serve as a measure of agreement in the magnitude of the lines of the genetic profile. The DRF was formed from a sum of the terms derived from the corresponding common genetic tests:

$$DRF = \frac{1}{n} \sum_i c - | LDU_{ij_a} - LDU_{ij_b} | \qquad (3)$$

where n is the total number of common tests, and c is a constant. The constant c was arbitrarily assigned a value of 3.0 in these calculations, to yield largely positive values for DRF. A DRF value of 2.0 corresponds to an average dose difference of one logarithmic unit. By the same procedure, as second dose-related function (DRF_c) was determined solely from the concordant common tests results.

Further details on the methodology for representation and analysis of genetic profiles may be found in Garrett et al. (1984).

Results

The data for the 65 pesticides can be subdivided initially into 3 main categories based on

qualitative results. These 3 categories and the CAS numbers and structures of the pesticides are shown in Tables 2, 3 and 4. The first category consists of pesticides that displayed largely positive results. These 9 agents, for the most part, were active in the in vitro and in some in vivo assays (Table 2). These compounds included: the organophosphate insecticides, acephate, demeton, monocrotophos, trichlorfon; the phthalimide fungicides, captan and folpet; and the thiocarbamate herbicides, diallate, sulfallate and triallate.

The next major category contained 26 chemicals that gave positive results in a smaller number of tests, and a few chemicals which were not fully evaluated (Table 3). This second category can be divided into two parts, one of which contains either halogenated aromatic or heterocyclic halogenated ring structures. There are 12 of these halogenated compounds, three of which are the phenoxy herbicides, 2,4-D, 2,4-DB and 2,4,5-T. The remaining part of the second category consists of the organophosphate insecticides, azinphosmethyl, crotoxyphos, disulfoton, methyl parathion; the ethylenebisdithiocarbamate fungicides, maneb, mancozeb and zineb; the pyrethroid insecticides, allethrin, chrysanthemic acid and ethyl chrysanthemate; and 4 more structurally diverse compounds, benomyl, cacodylic acid, dinoseb and *sec.*-butylamine.

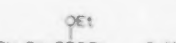
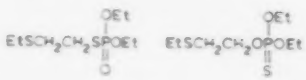
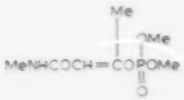
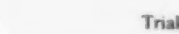
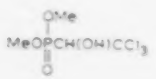
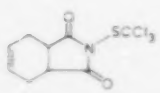
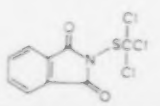
The third category of pesticides gave negative results in all tests in which they were evaluated. This category consisted of 30 chemicals from diverse chemical classes. These chemicals are listed in Table 4.

Pesticides with largely positive results

8 of the 65 pesticides were positive for point/gene mutation in prokaryotic and eukaryotic systems and caused DNA damage in eukaryotes. Their genetic activity profiles are shown in Figs. 2, 3 and 4. Four of the agents are organophosphate insecticides that display rather

TABLE 2

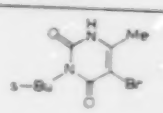
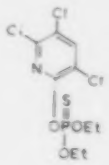
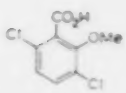
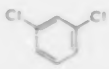
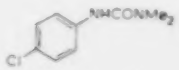
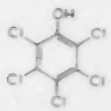
CHEMICAL STRUCTURE AND GROUPS OF PESTICIDES IN CATEGORY I

Chemical	CAS No.	Structure	Chemical	CAS No.	Structure
Organophosphate Insecticides			*Thiocarbamate Herbicides*		
Acephate(I)	30560-19-1		Diallate(H)	2303-16-4	
Demeton(I)	8065-48-3		Sulfallate(H)	95-06-7	
Monocrotophos(I)	6923-22-4		Triallate(H)	2303-17-5	
Trichlorfon(I)	52-68-6				
Phthalimide Fungicides					
Captan(F)	133-06-2				
Folpet(F)	133-07-3				

extensive genotoxic activity (Fig. 2). Of the test results for these 4 compounds, 46–75% were positive. The 2 phthalimide fungicides, captan and folpet, yielded more than 3 times as many positive as negative test results (Fig. 3). Each of these compounds contains a (trichloromethyl)thio group. 3 thiocarbamate herbicides, diallate, triallate and sulfallate were somewhat less active (Fig. 4). 29–63% of the tests gave positive results for these 3 compounds. All 3 compounds were positive, in Salmonella tester strains TA97 (SA9) and TA100 (SA0). Diallate and triallate gave positive results in the *S. cerevisiae* D3 mitotic recombination assay (YE3) and in the mouse lymphoma forward mutation assay (L5T) The genetic activity profiles of diallate and triallate were almost identical (Fig. 4). Diallate gave positive results as a germ cell mutagen in the Drosophilia sex-linked lethal test (SRL), but triallate was negative. Thus, it is possible that a structural difference of only one chlorine atom may have conferred genetic activity or inactivity in the SRL test. The third compound,

TABLE 3

CHEMICAL STRUCTURE AND GROUPS OF PESTICIDES IN CATEGORY II

Chemical	CAS No.	Structure
Halogenated Compounds		
Bromacil(H)	314-40-9	
Chlorpyrifos(I)	2921-88-2	
Dicamba(H)	1918-00-9	
m-Dichlorobenzene(I)	541-73-1	
o-Dichlorobenzene(I)	95-50-1	
Monuron(H)	150-68-5	
Pentachlorophenol(H)	87-86-5	

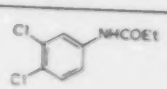
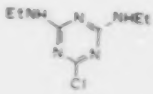
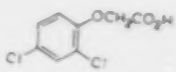
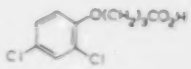
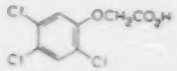
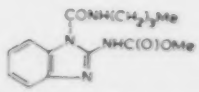
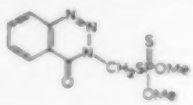
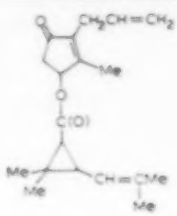
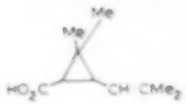
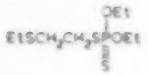
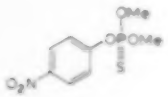
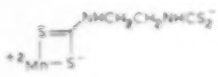
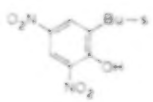
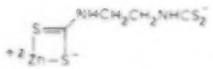
Chemical	CAS No.	Structure
Halogenated Compounds (Cont'd.)		
Propanil(H)	709-98-8	
Simazine(H)	122-34-9	
2,4-D(H)	94-75-7	
2,4-DB(H)	94-82-6	
2,4,5-T(H)	93-76-5	
Carbamate fungicide		
Benomyl(F)	17804-35-2	

TABLE 3(continued)

Chemical	Cas No.	Structure	Chemical	CAS No.	Structure
Organophosphate Insecticides			*Pyrethroid Insecticides*		
Azinphos-methyl(I)	86-50-0		Allethrin(I)	584-79-2	
Crotoxyphos(I)	7700-17-6		Chrysanthemic acid(I)	10453-89-1	
Disulfoton(I)	298-04-4		Ethyl chrysanthemate(I)	97-41-6	
Methyl parathion(I)	298-00-0				
Ethylenebisdithiocarbamates			*Structurally Diverse Pesticides*		
Mancozeb(F)	8018-01-7	$C_4H_6MnN_2S_6 \cdot C_4H_6N_2S_4Zn$	Cacodylic acid(H)	75-60-5	$Me_2AsOH:O$
Maneb(F)	12427-38-2		Dinoseb(H)	88-85-7	
Zineb(F)	12122-67-7		sec.-Butylamine(F)	13952-84-6	$s-BuNH_2$

sulfallate, gave positive results only in the Salmonella assay and was not evaluated in the YEH, YER, L5T or SRL test systems. Both sulfallate and diallate are considered animal carcinogens (Griesemer and Cueto, 1981; Innes et al., 1969).

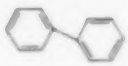
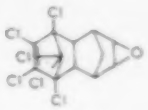
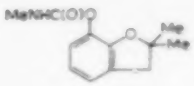
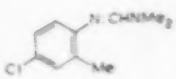
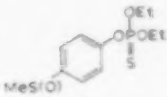
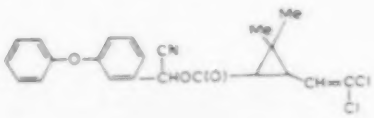
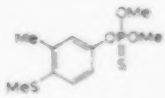
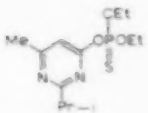
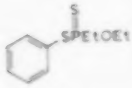
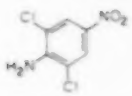
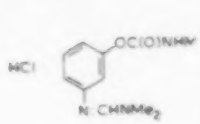
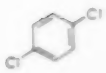
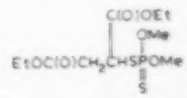
Pesticides with fewer positive results

The genetic activity profiles of an additional group of 7 pesticides are shown in Fig. 5 and 6. These compounds were positive for gene mutation and DNA damage or chromosomal effects only in eukaryotic systems. The compounds included four organophosphate insecticides, azinphos-methyl, crotoxyphos, disulfoton and methyl parathion (Fig. 5); and 3 structurally diverse pesticides, cacodylic acid, monuron and benomyl (Fig. 6). Cacodylic acid and monuron are herbicides and benomyl is a fungicide.

The genetic effects of azinphos-methyl, crotoxyphos, methyl parathion and cacodylic acid were

TABLE 4

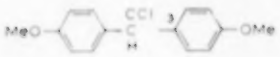
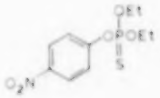
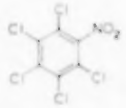
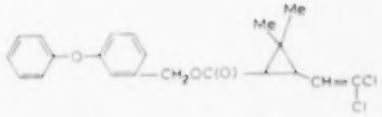
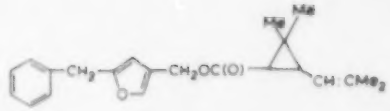
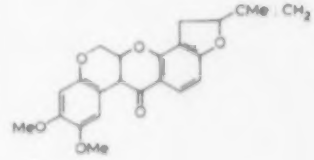
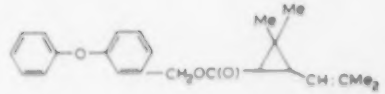
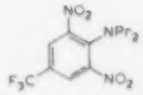
CHEMICAL STRUCTURE AND GROUPS OF PESTICIDES IN CATEGORY III

Chemical	CAS No.	Structure	Chemical	CAS No.	Structure
Negative Pesticides			*Negative Pesticides* (Cont'd.)		
Aspon(I)	3244-90-4		DSMA(H)	144-21-8	
Biphenyl(F)	92-52-4		Endrin(I)	72-20-8	
Carbofuran(I)	1563-66-2		Ethion(I)	563-12-2	
Chlordimeform(I)	6164-98-3		Fensulfothion(I)	115-90-2	
Cypermethrin(I)	52315-07-8		Fenthion(I)	55-38-9	
Diazinon(I)	333-41-5		Fonofos(I)	944-22-9	
Dichloran(F)	99-30-9		Formetanate(I)	23422-53-9	
p-Dichlorobenzene	106-46-7		Malathion(I)	121-75-5	

detected initially in the *S. cerevisiae* D3 assay (YE3). Disulfoton was positive in the human lung fibroblast unscheduled DNA synthesis assay (UDH). These pesticides were positive only in eukaryotic systems and, except for benomyl, were tested thoroughly in prokaryotic systems with negative results.

The genetic activity profiles of azinphos-methyl, crotoxyphos, disulfoton and methyl parathion, are similar as can be seen by inspection of Fig. 5. All 4 of these insecticides were positive in the L5T test system. Crotoxyphos is a phosphoric acid ester and is thus in the same chemical class as the Category 1 compounds acephate, monocrotophos

TABLE 4 (continued)

Chemical	CAS No.	Structure
Negative Pesticides (Cont'd.)		
Methomyl(I)	16752-77-5	MeNHC(O)ON:C(SMe)Me
Methoxychlor(I)	72-43-5	
MSMA(H)	2163-80-6	$MeAs(OH)_2O \cdot Na$
Parathion(I)	56-38-2	
PCNB(F)	82-68-8	
Permethrin(I)	52645-53-1	
Phorate(I)	298-02-2	
Polyram(H)	9006-42-2	NOT AVAILABLE
Resmethrin(I)	10453-86-8	
Rotenone(I)	83-79-4	
sec.-Butylamine H_3PO_4(F)	28508-28-3	$s-BuNH_2 \quad H_3PO_4$
Siduron(H)	1982-49-6	
Sumithrin(I)	26002-80-2	
Trifluralin(H)	1582-09-8	

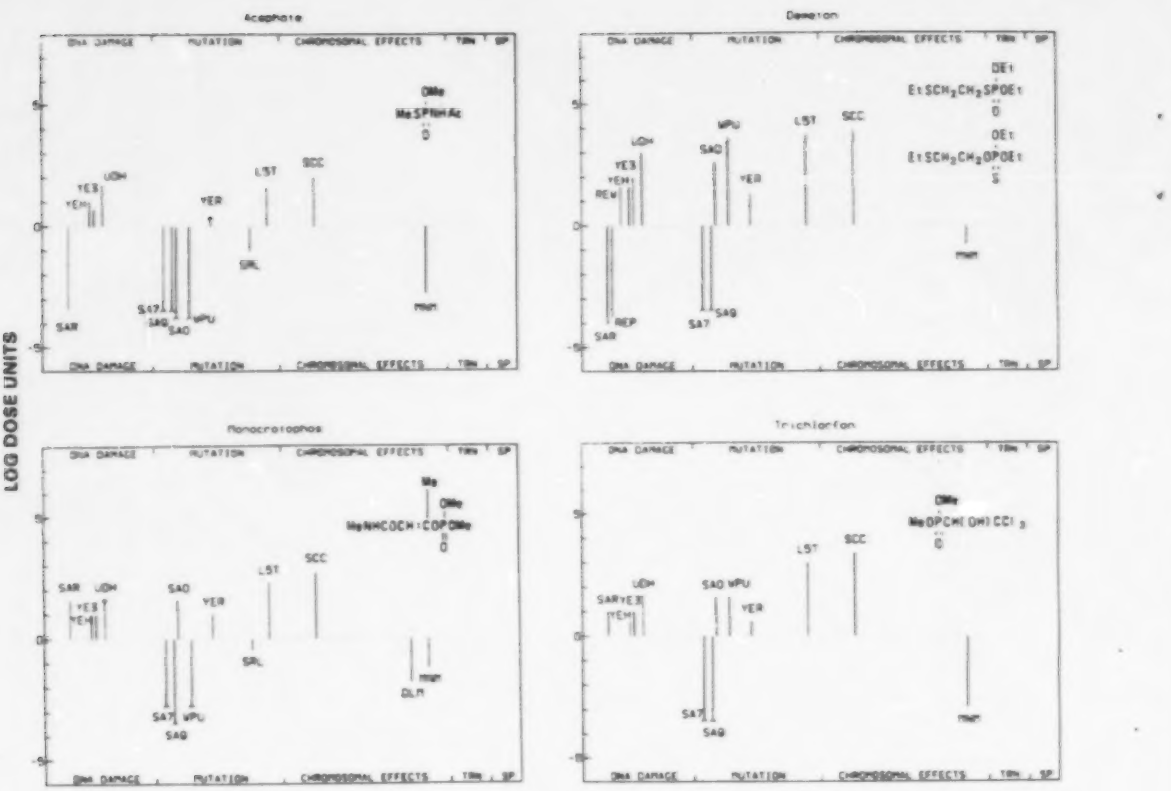


Fig. 2. Genetic activity profiles for 4 organophosphate insecticides. The symbol Λ is used to denote the use of an exogenous metabolic system.

and trichlorfon, but it displays a substantially narrower range of genetic activity.

The profile of disulfoton is the most unique of the 4 organophosphate insecticides shown in Fig. 5. Disulfoton is positive in UDH and negative in YE3 unlike the other 3 chemicals. Disulfoton and azinphos-methyl are both dithiophosphate ester insecticides. The genetic activity profiles of 6 such compounds are shown in Fig. 7. These compounds were not sufficiently active to be grouped with the Category 2 insecticides. Disulfoton, the most frequently positive of the dithiophosphate esters, elicited gene mutation, primary DNA damage and chromosomal effects. Interestingly, disulfoton and the structurally similar compound phorate (which was negative in the initial test battery) resemble the frequently positive agents distinguished by unbranched side chains without ring structures (e.g., demeton). Phorate has not been tested in 2 (L5T and SCC) of the 3 assays for which disulfoton was positive. Based on an analysis of insecticide performance in a number of common bioassays, we previously included disulfoton in a group of chemicals with azinphos-methyl, crotoxyphos and methyl parathion (Waters et al., 1982). Disulfoton may exert its effects by a reaction mechanism somewhat similar to that of demeton.

Methyl parathion is an organothiophosphate ester that could be classified with the insecticides, demeton and chlorpyrifos. Both methyl parathion and chlorpyrifos are considerably less active than demeton. A possible mechanism to explain the disparity in activity of demeton as compared to the other organothiophosphate esters is discussed

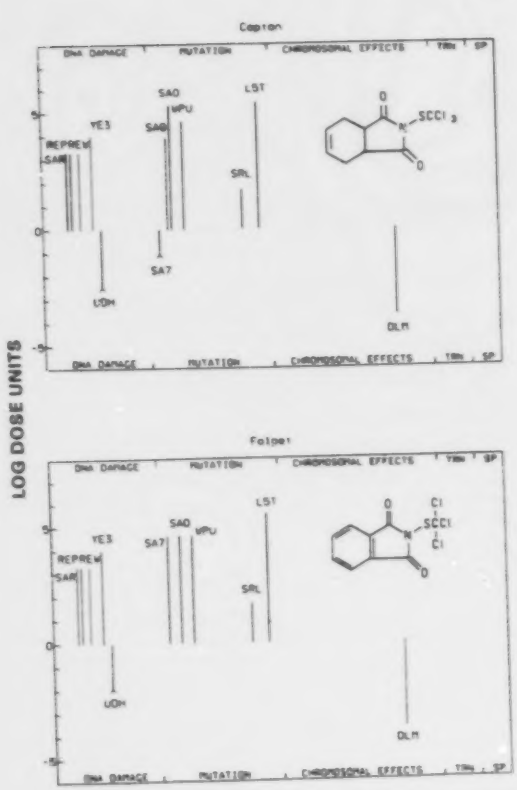


Fig. 3. Genetic activity profiles for 2 phthalimide fungicides. The symbol Λ is used to denote the use of an exogenous metabolic system.

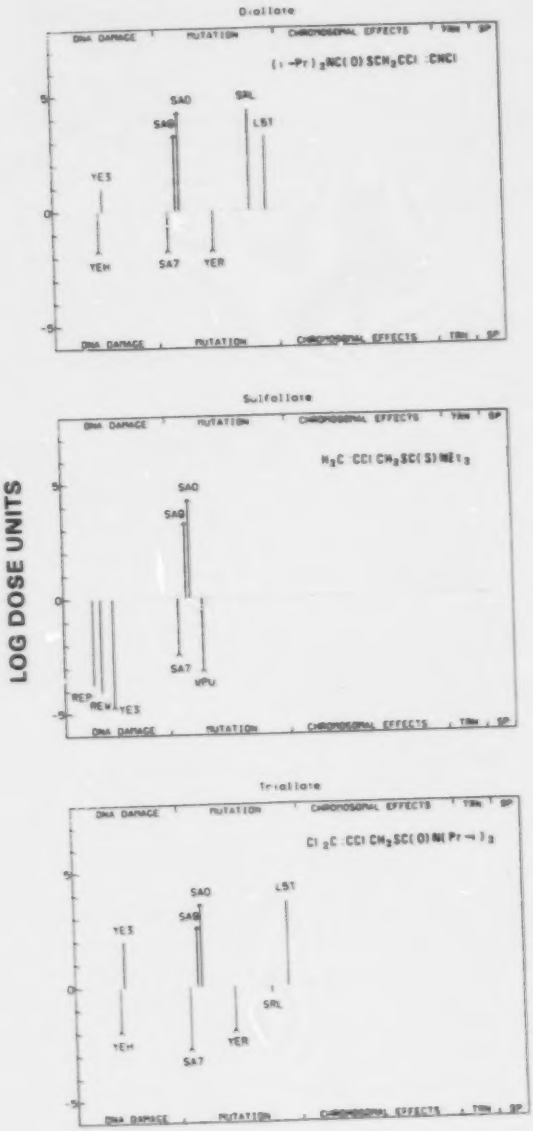


Fig. 4. Genetic activity profiles for 3 thiocarbamate herbicides. The symbol Λ is used to denote the use of an exogenous metabolic system.

later. In Fig. 8 the genetic activity profiles of methyl parathion and chlorpyrifos are compared with a number of other thiophosphoric acid insecticides that were negative in the initial test battery (cf. Table 1). It should be noted that these largely negative compounds could prove positive for chromosomal effects or point/gene mutation in eukaryotes if evaluated further. In this regard, parathion should be evaluated in L5T and SCC since the structurally similar compound, methyl parathion, was positive in these test systems.

As shown in Fig. 6, cacodylic acid, monuron and benomyl were also positive in eukaryotic systems. Benomyl and monuron both gave positive results for point/gene mutation in the L5T system and positive results for chromosomal effects in the SCC and MNM tests. Cacodylic acid, an organoarsenical, caused gene mutation, DNA damage and chromosomal effects. Two other

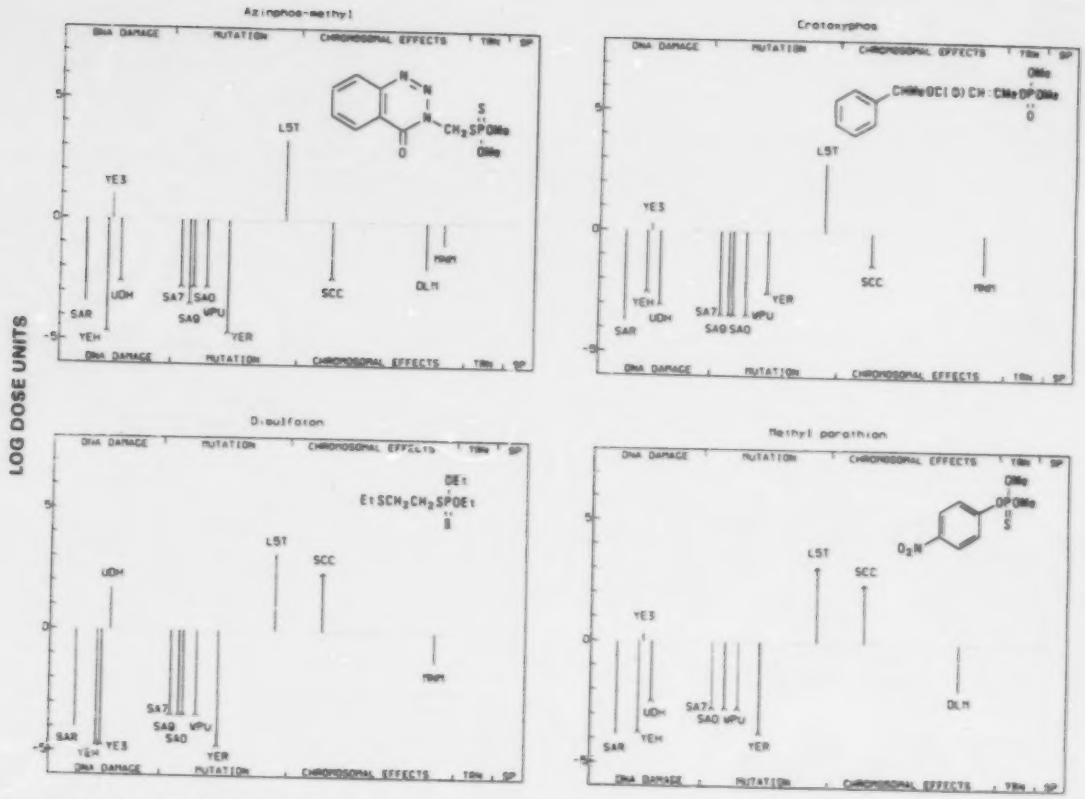


Fig. 5. Genetic activity profiles for 4 organophosphate insecticides. The symbol Λ is used to denote the use of an exogenous metabolic system.

chemicals, bromacil and simazine, were also positive only in eukaryotes, and specifically in the L5T and SRL gene mutation tests. See Fig. 9.

3 chemicals, allethrin, chrysanthemic acid and ethyl chrysanthemate gave positive results in only one test system: SA0, REW and YE3, respectively (Fig. 10). These chemicals are members of a group of pyrethroids that were generally negative in the initial test battery (cf. Table 1). The pyrethroids are natural plant products or their synthetic analogues.

A unique group of pesticides caused DNA damage only in prokaryotic systems, although testing for gene mutation and chromosomal damage is not complete. This group includes chrysanthemic acid, the herbicide dinoseb, and the fungicide *sec.*-butylamine. *sec.*-Butylamine gave positive results only in the REP test system. Dinoseb gave positive results in the REP, REW and SAR assays (Fig. 11) and its genetic activity profile was very similar to that of chlorpyrifos. Dinoseb was negative in the SRL test system. The remaining 9 chemicals that displayed only DNA damage were all chlorinated aromatics. Their genetic activity profiles and chemical structures are shown in Fig. 12. These compounds may cause DNA damage by nucleophilic aromatic substitution reactions. The phenoxy herbicides, 2,4-D, 2,4-DB and 2,4,5-T form a subgroup of the chlorinated compounds; evidence of their positive effects in eukaryotic systems has been published elsewhere (Zetterberg, 1977).

Although testing is incomplete, *m*-dichlorobenzene and pentachlorophenol were positive only for

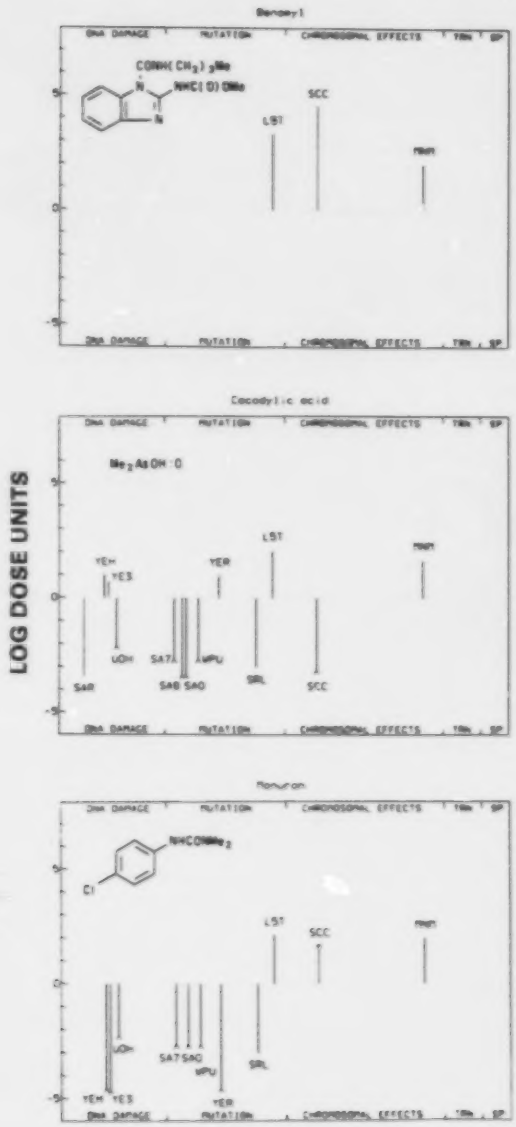


Fig. 6. Genetic activity profiles for 3 structurally diverse pesticides. The symbol Λ is used to denote the use of an exogenous metabolic system.

DNA damage in a prokaryotic and a eukaryotic system. 4 other pesticides (ethyl chrysanthemate, mancozeb, maneb and zineb) were positive for DNA damage only in a eukaryotic system. The genetic activity profiles for the last 3 chemicals are shown in Fig. 13. Since positive results obtained in the YE3 test were frequently confirmed by the L5T bioassay (Waters et al., 1982), these chemicals should be evaluated further in mammalian cell mutagenesis test systems.

Comparative computer analysis of the activity profiles

Qualitative aspects

The results presented in the previous section reveal some of the complexity of comparing and interpreting the results of genetic tests for a large number of structurally diverse compounds. As we have demonstrated, a simple classification can often be made by visual inspection of the profiles and corresponding chemical structures. It was of interest to make a step-by-step pairwise comparison of chemicals using the computer to identify compounds displaying similar genetic activity. Correlations in test results were examined using the data analysis techniques previously reported (Garrett et al., 1984). These techniques, summarized under Methods, employ pattern recognition to identify similar genetic activity profiles. The data were examined for (1) all common bioassays performed for each pair of chemicals and (2) for qualitative and quantitative agreement of individual profile lines.

From the computer-generated profiles for the 65 pesticides, 2080 pairwise chemical combinations are possible. The significant chemical combinations were determined by calculating the probability (P) that the number of concordant test results would occur by chance. This calculation is based on eqn. 2.

The matching process for the pesticides was performed by first selecting those pesticides with 2 or more positive test results. This criterion reduced the number of possible pairwise chemical combinations to 190. Profile matches at $P \leq 0.02$ and with a minimum of 2 positive concordant tests were selected from the possible combinations (Table 5). Frequently, the ratio of concordant to conflicting test results was highly significant ($P \leq 0.01$) such that a match in the profiles for a pair of compounds indicated common genetic activity. Using these criteria, no statistically significant

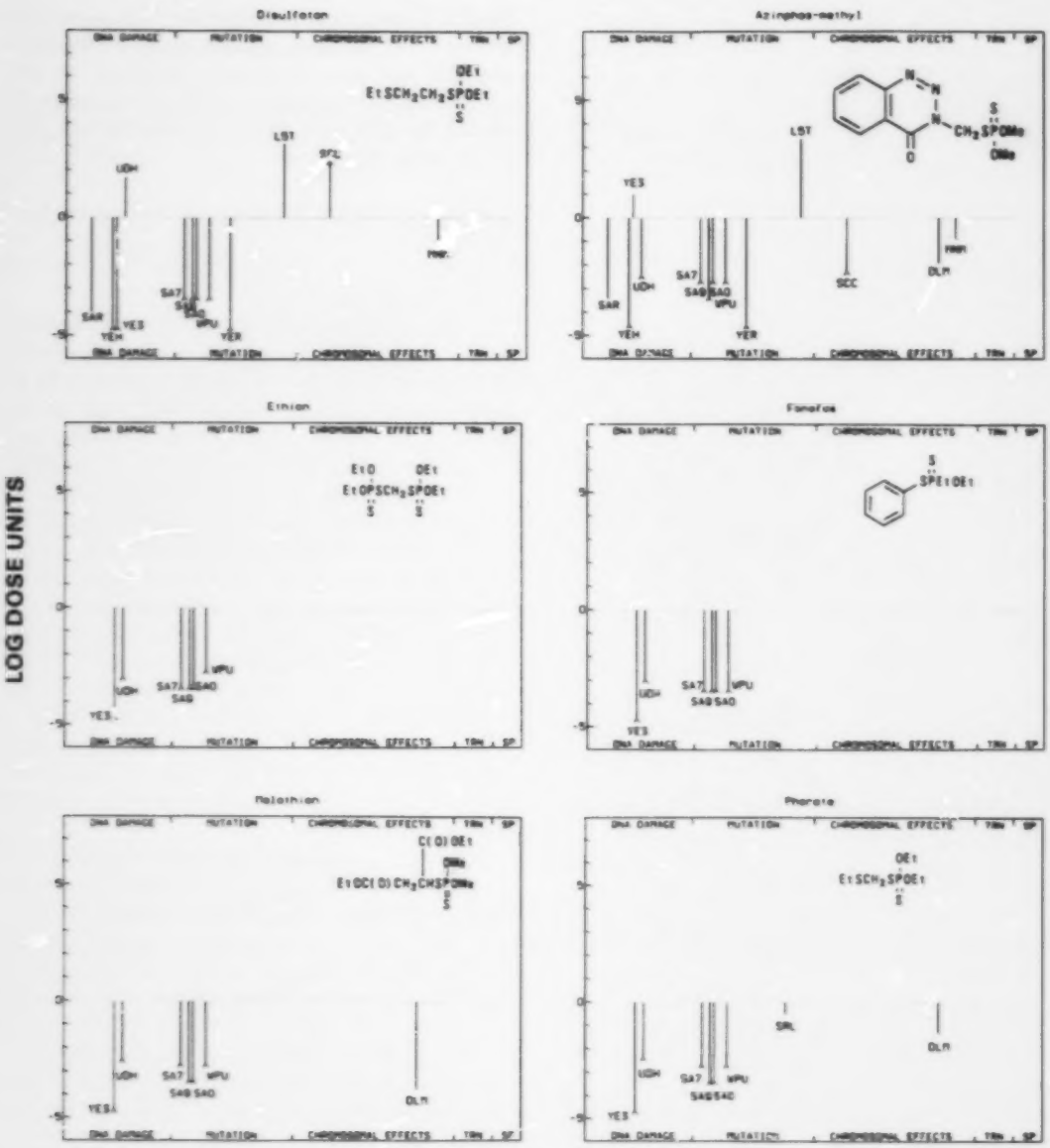


Fig. 7. Genetic activity profiles for 6 dithiophosphate ester insecticides. The symbol Λ is used to denote the use of an exogenous metabolic system.

correlation was found in the profiles for 45 of the 65 compounds. Only 20 pairings showed agreement with a number of tests greater than that which could be expected by chance (Table 5). For these selected combinations, the number of bioassays used in comparing any 2 chemicals ranged from 6 to 13 with a mean of 10. The number of conflicting or discordant common tests was fre-

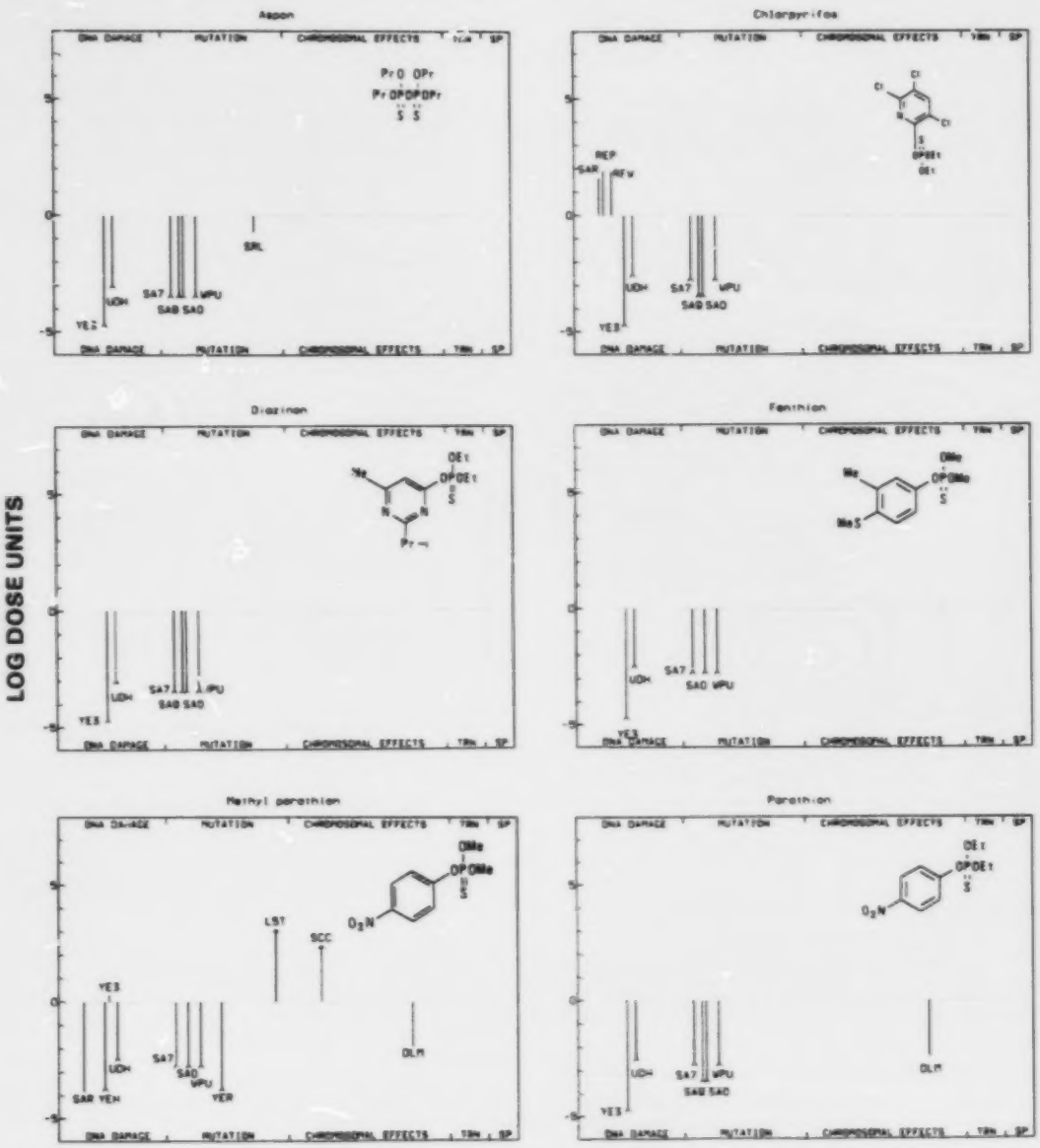


Fig. 8. Genetic activity profiles for 6 organophosphate insecticides. The symbol Λ is used to denote the use of an exogenous metabolic system.

quently 0 or 1 and the mean was 0.8.

The data shown in Table 5 indicating the significant relationships between chemical pairs can be used to arrange these pesticides into groups or sets of chemicals displaying similar genetic activity and chemical structures. This process can be facilitated by the use of elementary set theory and Venn diagrams (Fig. 14). Each circle of the Venn

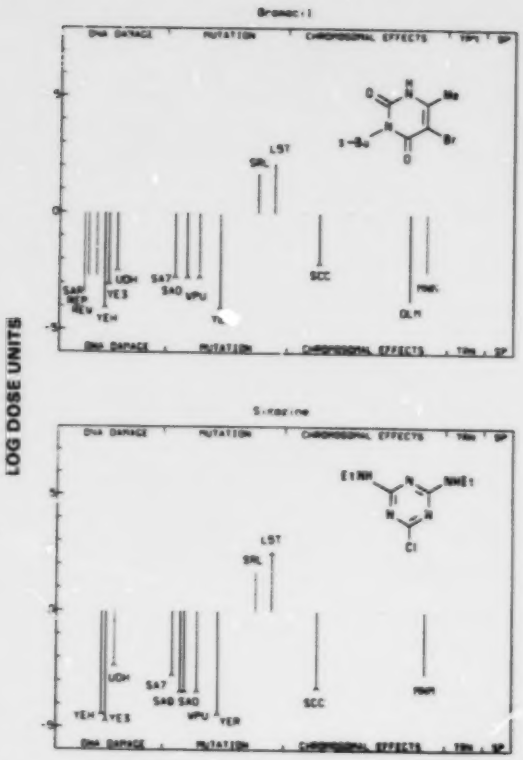


Fig. 9. Genetic activity profiles for bromacil and simazine. The symbol Λ is used to denote the use of an exogenous metabolic system.

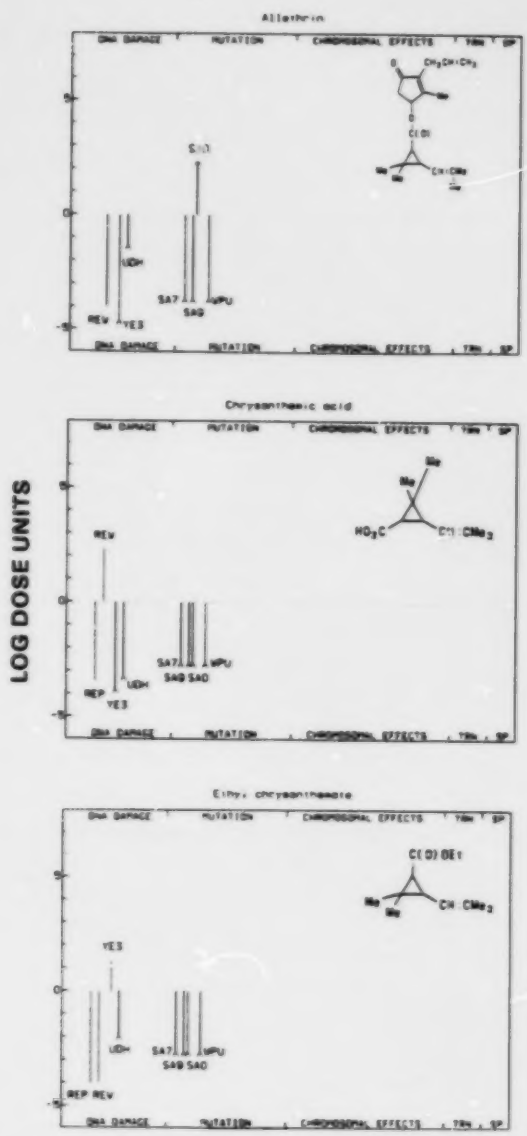


Fig. 10. Genetic activity profiles for 3 pyrethroids. The symbol Λ is used to denote the use of an exogenous metabolic system.

diagram is equivalent to the total number of elements of the set and can represent the totality of genetic activities or corresponding chemical structures. The intersection of two sets (chemicals) represents common genetic activity or chemical structure.

By examining the data in Table 5, six groups of compounds could be identified. 3 of the 6 groups are simple pairs of structurally similar pesticides: captan and folpet, diallate and triallate, and bromacil and simazine. The combined genetic activity for these three pairs are shown in Figs. 15, 16 and 17.

3 of the chemical groups revealed more complex patterns of common genetic activity (Fig. 14). In Fig. 14A, a Venn diagram for acephate, demeton, monocrotophos and trichlorofon is shown. The combined activity profiles for this group of pesticides are shown in Fig. 18. Inspection of Fig. 18 shows that the 4 pesticides are related in their genetic activity, but use of the Venn diagrams more readily allows separation of similar or dis-

TABLE 5

COMPARISONS OF GENETIC ACTIVITY PROFILES FOR SELECTED PAIRS OF PESTICIDES

Chemical	Number of bioassays		Probability value (P)	DRF	DRF_c
	Common (n) tests	Conflicting (m) results			
Acephate					
Demeton	12	2	0.019	1.0	1.9
Mancozeb	6	0	0.016	1.2	1.2
Maneb	6	0	0.016	1.2	1.2
Monocrotophos	13	2	0.011	1.7	2.4
Azinphos-methyl					
Crotoxyphos	12	0	0.000	2.2	2.2
Methyl parathion	11	1	0.006	2.3	2.7
Simazine	11	1	0.006	2.0	2.4
Bromacil					
Simazine	11	0	0.001	2.5	2.5
Captan					
Folpet	11	1	0.006	2.4	2.9
Chlorpyrifos					
Dinoseb	8	0	0.004	2.5	2.5
Propanil	9	1	0.020	1.8	2.3
Dicamba	9	1	0.020	2.0	2.6
Crotoxyphos					
Azinphos-methyl	12	0	0.000	2.2	2.2
Methyl parathion	10	1	0.011	2.1	2.4
Demeton					
Monocrotophos	12	2	0.019	1.3	2.2
Trichlorfon	12	1	0.003	1.7	2.1
Acephate	12	2	0.019	1.0	1.9
Diallate					
Triallate	8	1	0.035	1.9	2.3
Dicamba					
Chlorpyrifos	9	1	0.020	2.0	2.6
Dinoseb	9	1	0.020	1.7	2.2
Dinoseb					
Chlorpyrifos	8	0	0.004	2.5	2.5
Dicamba	9	1	0.020	1.7	2.2
Folpet					
Captan	11	1	0.006	2.4	2.9
Mancozeb					
Maneb	8	0	0.004	2.8	2.8
Acephate	6	0	0.016	1.2	1.2
Maneb					
Mancozeb	8	0	0.004	2.8	2.8
Acephate	6	0	0.016	1.2	1.2
Methyl parathion					
Monuron	9	1	0.020	2.1	2.6
Crotoxyphos	10	1	0.011	2.1	2.4
Azinphos-methyl	11	1	0.006	2.3	2.7

TABLE 5 (continued)

Chemical	Number of bioassays		Probability value (P)	DRF	DRF_c
	Common (n) tests	Conflicting (m) results			
Monocrotophos					
Acephate	13	2	0.011	1.7	2.4
Demeton	12	2	0.019	1.3	2.2
Trichlorfon	12	1	0.003	2.2	2.5
Monuron					
Methyl parathion	9	1	0.020	2.1	2.6
Propanil					
Chlorpyrifos	9	1	0.020	1.8	2.3
Simazine					
Azinphos-methyl	11	1	0.006	2.0	2.4
Bromacil	11	0	0.001	2.5	2.5
Triallate					
Diallate	8	1	0.035	1.9	2.3
Trichlorfon					
Demeton	12	1	0.003	1.7	2.1
Monocrotophos	12	1	0.003	2.2	2.5

[a] Results of genetic activity profile matches with a minimum of two positive concordant tests. Values except those for diallate and triallate are shown only if $P \leq 0.02$.

similar genetic effects. As shown in the Venn diagram, Fig. 14A, the 4 compounds, acephate, demeton, monocrotophos and trichlorfon, gave positive results for DNA damage in YEH, YE3 and UDH and for mutation in the YER and L5T systems, but gave negative results for mutation in SA7 and SA9. These pesticides also were positive for chromosomal effects in SCC but were negative in the micronucleus assay (MNM). Trichlorfon, monocrotophos and demeton gave positive results for base-pair substitution in the SA0 system. This activity is indicated by the intersection of the circles representing the 3 compounds in Fig. 14A. Common activity for demeton and trichlorfon was demonstrated by mutation in the WPU test system. Trichlorfon and monocrotophos both produced differential toxicity in *S. typhimurium* (SAR) while demeton and acephate were negative in the same assay. Monocrotophos and acephate gave negative results in the WPU and SRL mutation systems. Maneb and Mancozeb (not shown in Fig. 14) had common negative results with acephate in both the SAR and WPU test systems, and had 4 additional test results in common with the 4 organophosphate insecticides; e.g., positive results in YE3 and UDH, negative results in SA7 and SA9.

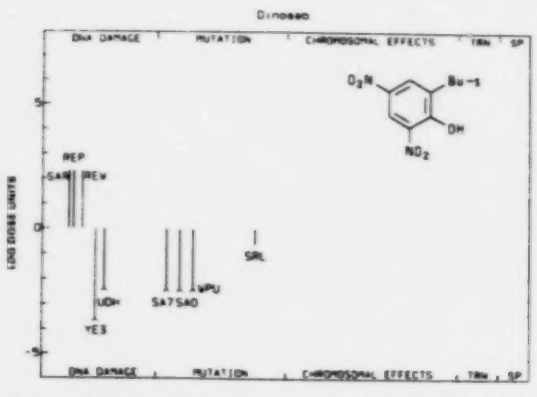


Fig. 11. Genetic activity profile for dinoseb. The symbol Λ is used to denote the use of an exogenous metabolic system.

The combined genetic activity profile for an additional 4 pesticides, azinphos-methyl, crotoxyphos, methyl parathion and monuron which form a group is shown in Fig. 19. The correspond-

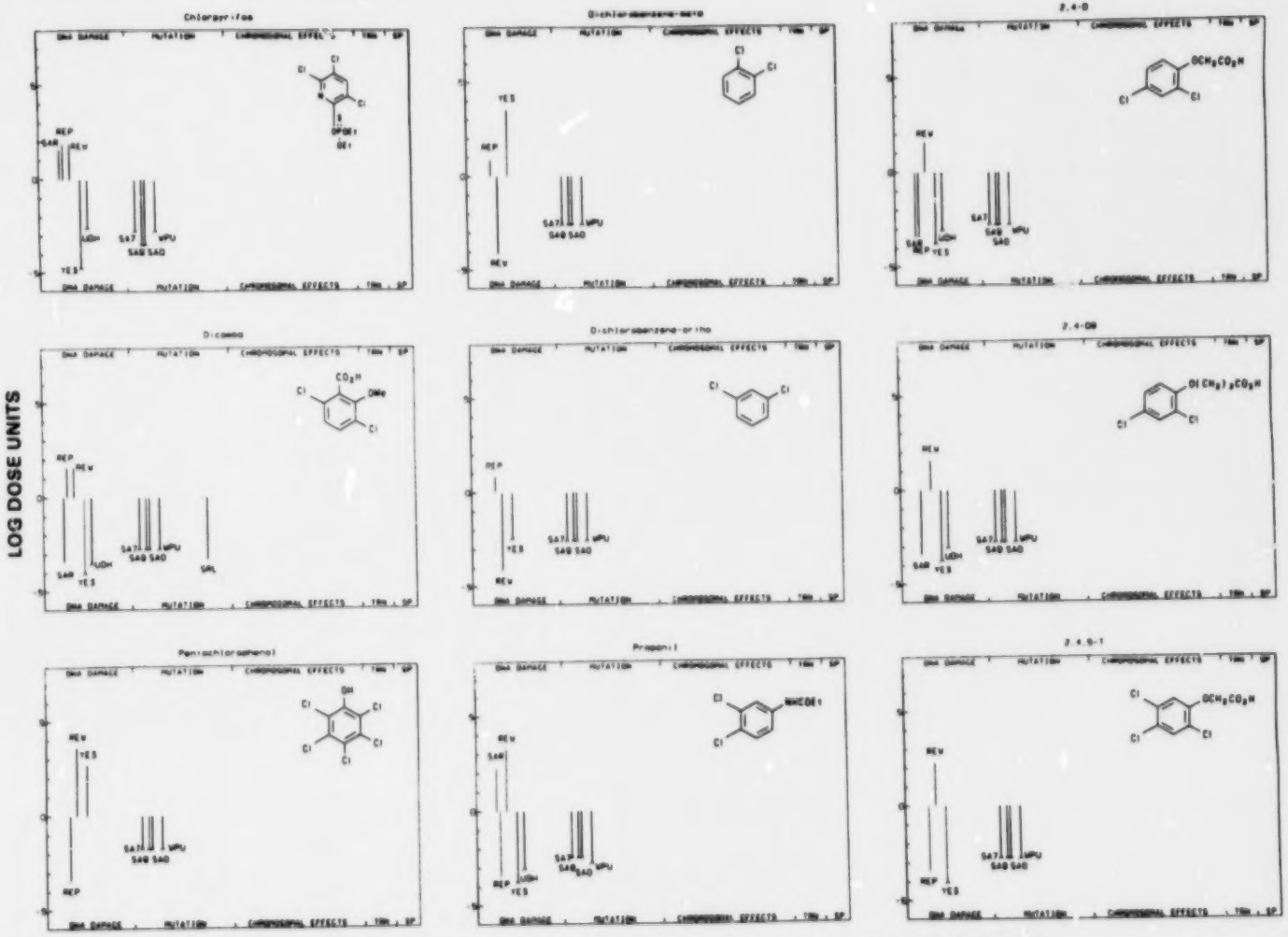


Fig. 12. Genetic activity profiles for 9 chlorinated aromatics. The symbol Λ is used to denote the use of an exogenous metabolic system.

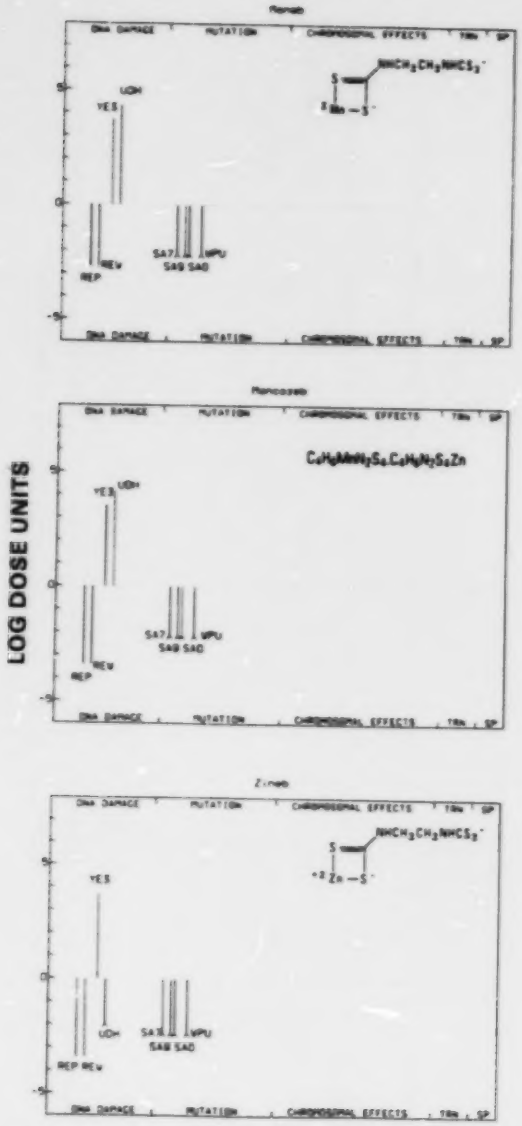


Fig. 13. Genetic activity profiles for 3 ethylene bisdithiocarbamate fungicides. The symbol A is used to denote the use of an exogenous metabolic system.

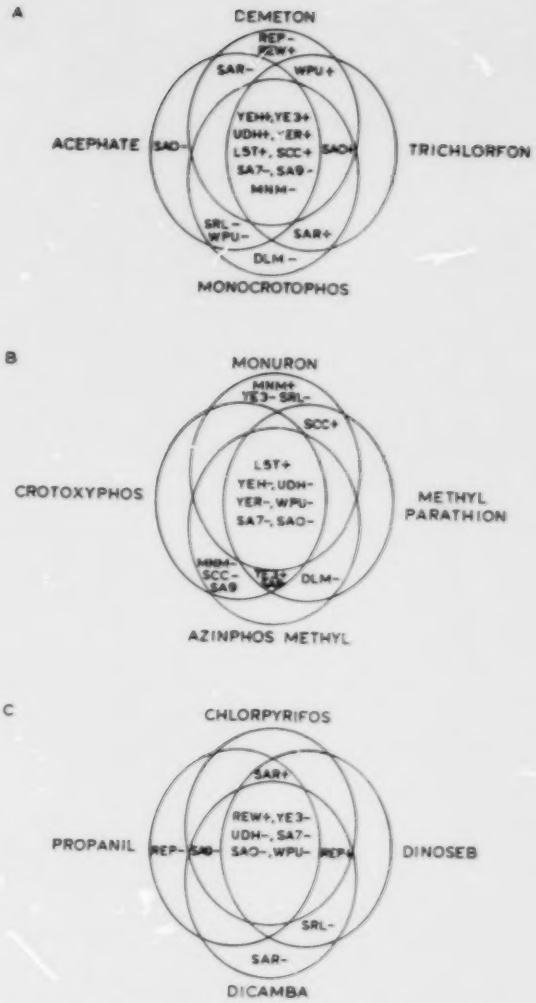


Fig. 14. Venn diagrams illustrating common test results for 3 groups of chemicals. A circle represents a given chemical compound, and within each circle are the tests codes and corresponding positive or negative responses for that compound. The intersection of two or more circles shows common test results for each of the represented compounds.

ing Venn diagram is shown in Fig. 14B. These 4 pesticides gave negative results in the YEH, UDH, SA7, SA0, WPU, and YER test systems and positive results in L5T. Monuron was negative in the YE3 test; however, the other 3 compounds were positive in YE3 but negative in SAR. Azinphos-methyl and crotoxyphos were negative in the SA9 and SCC tests, and azinphos-methyl and methyl parathion were negative in the DLM system.

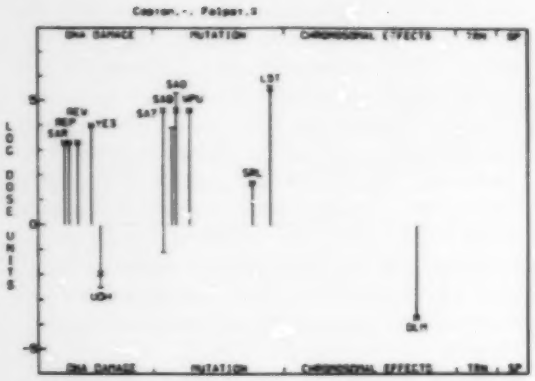


Fig. 15. Combined activity profiles for captan and folpet.

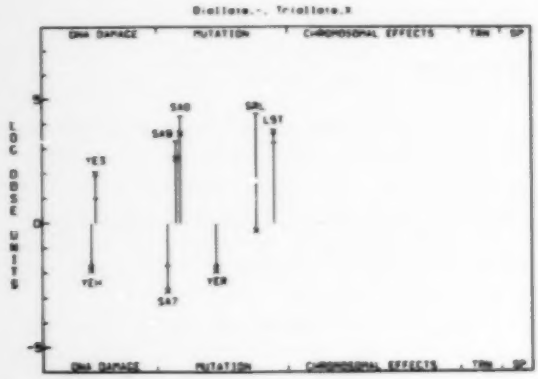


Fig. 16. Combined activity profiles for diallate and triallate.

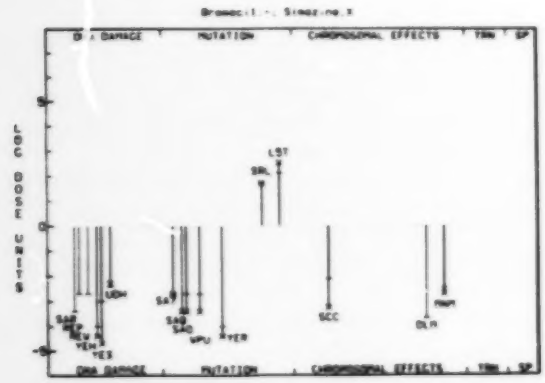


Fig. 17. Combined activity profiles for bromacil and simazine.

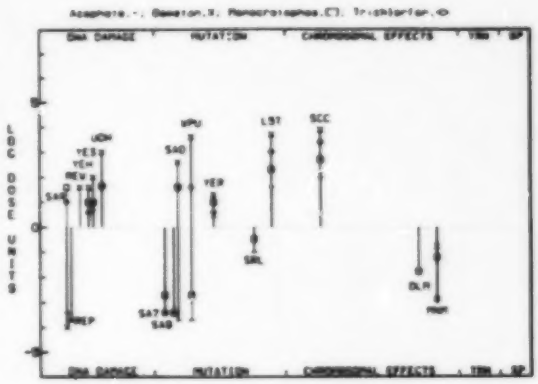


Fig. 18. Combined activity profiles for acephate, demeton, monocrotophos and trichlorfon.

Methyl parathion and monuron had common positive activity in SCC.

The remaining pesticides that formed a statistically significant group consisted of 4 agents that produce DNA damage only in eukaryotes: dinoseb, propanil, chlorpyrifos and dicamba. The combined activity profiles are shown in Fig. 20. The corresponding Venn diagram is shown in Fig. 14C. Results common to all 4 pesticides included positive results in the REW system and negative results in YE3, UDH, SA7, SA0 and WPU. All the compounds except propanil were positive in REP, and all compounds except dicamba were positive in SAR. Dinoseb and dicamba gave nega-

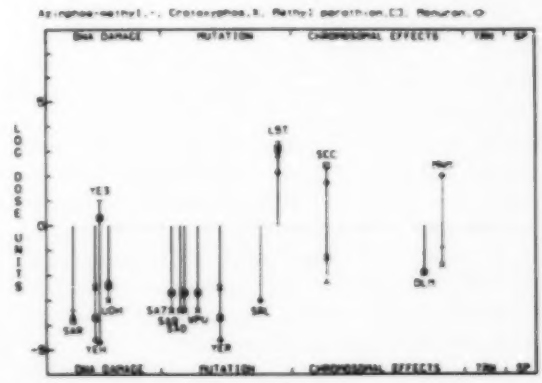


Fig. 19. Combined activity profiles for azinphos-methyl, crotoxyphos, methyl parathion and monuron.

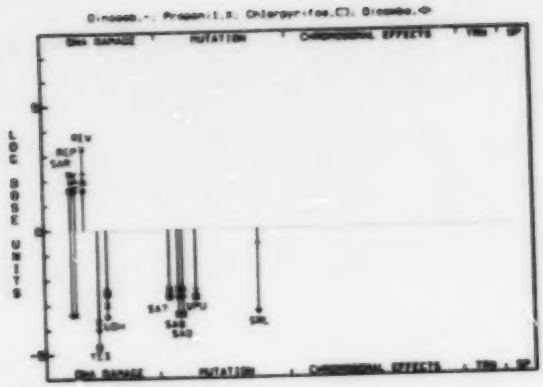


Fig. 20. Combined activity profiles for dinoseb, propanil, chlorpyrifos and dicamba.

tive results in SRL. Dinoseb was not tested in SA9 but the other 3 compounds were negative in SA9.

In summary, the 3 major groups of pesticides identified by the qualitative matching procedure were those causing: (1) DNA damage, gene mutation, and chromosomal effects, (2) mutation in mammalian cells and chromosomal effects, and (3) DNA damage only.

Quantitative aspects

A quantitative measurement of overall agreement of effective or ineffective dose levels for common bioassays for any pair of chemicals can be calculated as the dose-related function (DRF). See Table 5. A value ≥ 2 indicates that the effective or ineffective doses for common bioassays agree within an order of magnitude, or one logarithmic unit. Many of the values for the DRF were approximately 2, indicating substantial quantitative agreement in the responses of common test systems. The mean value of the DRF (values shown in Table 5) was 1.9 with a standard deviation of 0.5. The index of dose as a function of only the concordant tests (DRF_c) averaged 2.3 with standard deviation of 0.4. DRF_c may provide a better measure of the quantitative agreement between similar profiles, since only common concordant pairs of tests are considered.

Certain quantitative aspects of the data may be cited to amplify the quantitative aspects of the profile matches for the 20 statistically significant combinations of pesticides. For example, the simple pairs of structurally similar pesticides displayed DRF_c values of 2.9, 2.5 and 2.5 for captan/folpet, diallate/triallate and simazine/bromacil, respectively. DRF_c values for the highly active agents trichlorfon/monocrotophos and maneb/mancozeb were 2.5 and 2.8, respectively. The group of organophosphate pesticides produced DRF_c values for azinphos-methyl/methyl parathion and methyl parathion/monuron of 2.7 and 2.6, respectively. Good agreement for the dose-related functions also was found for the chlorinated compounds. DRF_c values for dinoseb/chlorpyrifos and dicamba/chlorpyrifos were 2.5, 2.6, respectively. Hence, for these structurally similar compounds, there is very good evidence for quantitative as well as qualitative matching of genetic activity profiles.

Discussion

The present investigation makes use of genetic activity profiles to represent the qualitative bioassay results and quantitative information (LED or HID tested) in a two-dimensional configuration that facilitates rapid evaluation. The present pesticide data base is limited to test results obtained in one laboratory.

Three main categories of results were distinguished among the 65 compounds tested. 9 Category 1 pesticides were active in most of the in vitro and in vivo assays employed. 26 Category 2 pesticides gave fewer positive results and could be subdivided into 12 halogenated aromatic or heterocyclic ring compounds, a group of 4 organophosphate insecticides, 3 ethylenebisdithiocarbamate fungicides, 3 pyrethroid insecticides, and 4 structurally diverse compounds. Category 3 consisted of 30 pesticides which gave negative results in all tests.

We have been able to evaluate and classify these pesticides using 3 different techniques: (1) with respect to their overall genetic activity by inspection of the genetic activity profiles; (2) on the basis of their chemical structures, and (3) by means of computerized matching of the genetic activity profiles. The latter technique can be used to examine the data for all significant pairwise combinations of chemicals. Significant chemical

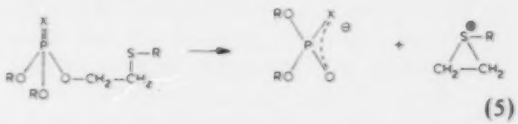
combinations were based on the calculation of the probability (P) that the number of concordant results would occur by chance. Thus, similarities in the profiles of genetic effects displayed by specific pairs of compounds can be determined and matches between pairs of profiles may be used to construct distinct groups of chemicals that display similar genetic activity. Venn diagrams also may be used to illustrate the pairwise combinations and the natural formation of groups of chemicals based on common genetic activities.

By forming the significant chemical combinations, insight may be gained as to the mechanisms of toxicity, provided all chemicals within a group contain the same reactive chemical moiety. In theory, the specific profile of genetic test results for particular reactive groups could thus be determined. The groups of chemicals formed by analysis of pesticide performance in a number of common bioassays were correlated with chemical structure and activity. Although pesticides within a group did not display identical profiles of genetic activity, components of the profiles matched other chemicals within the group.

4 of the most active pesticides that formed a group are the organophosphate insecticides: acephate, demeton, monocrotophos and trichlorfon. The major reactions of pentavalent phosphorous esters are phosphorylation and alkylation reactions. Although the phosphorylation reaction is responsible for the inhibition of the acetylcholinesterase and produces the corresponding neurotoxicity, certain biological activities of the phosphorous esters such as mutation appear to be due to the alkyation properties (Eto and Ohkawa, 1970). The general reaction mechanism is shown in eqn. 4 where N is a nucleophile (Eto, 1974).

(4)

The heterocyclic bases of nucleic acids may serve as nucleophilic agents for such a reaction, and alkylation may take place on nitrogen atoms possessing high electron density. The alkylation reaction is more likely with the methyl ester group than ethyl and higher alkyl esters groups, and phosphate esters are more reactive than the phosphorothionate esters (Eto, 1974). Thus, the reactivity of acephate, monocrotophos and trichlorfon may be explained by methylation reactions of the phosphate esters. Since demeton is an ethyl ester, this pesticle might act by a different mechanism. 2-Alkylthioethanols form cyclic ethylenesulfonium ions which are highly active alkylating agents. The reaction mechanism is:



(5)

and this mechanism may explain the genetic activity of demeton (Eto, 1974).

Among the more active agents in this analysis are the phthalimide fungicides, captan and folpet. These agents agents are also subject to nucleophilic attack, possibly at carbonyls or halogens prior to the ultimate scission of the N–S bond (Kohn, 1977).

The mutagenic activity of the thiocarbamate herbicides, diallate, sulfallate, and triallate may be due to the reactivity of sulfoxides which are formed metabolically (Casida et al., 1975; Schuphan et al., 1979). The metabolism of these compounds has been studied extensively and various chloroacrolein metabolites have been proposed as the ultimate mutagens (Schuphan et al., 1981).

Among pesticides with fewer positive genetic tests, computer analysis yielded a group of 4 pesticides, azinphos-methyl, crotoxyphos, methyl parathion and monuron. All 4 compounds were active only in eukaryotes. Monuron caused chromosomal breakage (micronuclei), and both monuron and methyl parathion induced sister-chromatid exchanges. The positive results for all 4 compounds in the L5T system may also reflect chromosomal damage (Moore et al., 1985). 3 members of this group of pesticides are organophosphate insecticides. Crotoxyphos is a phosphoric acid ester. Methyl parathion contains a thiophosphoric acid group, and azinphosmethyl contains a dithiophosphoric acid group. The thiocontaining compounds are not as potent alkylators as the phosphoric acid esters. Crotoxyphos is chemically similar to acephate, monocrotophos and trichlorfon, but possesses substantially less activity. Alkylating activity is directly related to

the electron affinity of the substituents (Eto, 1974).

One additional statistically significant group of pesticides consisted of 4 agents that produced DNA damage only in prokaryotes: dinoseb, propanil, chlorpyrifos and dicamba. All 4 chemicals gave positive results when damage was measured as differential toxicity in the REW system and all compounds except propanil were also positive in the REP system. Except for dinoseb, these compounds are chlorinated aromatics and may cause DNA damage by nucleophilic substitution reactions. In a previous report, we also included *o*-dichlorobenzene in a similar group of halogenated aromatics as well as the phenoxy herbicides 2,4-D, 2,4,-DB and 2,4,5-T (Waters et al., 1982).

In conclusion in the present analysis using the profile matching methodology we selected from 2080 different chemical combinations of the 65 pesticides only 20 statistically significant pairwise combinations. Moreover, by arranging these pesticide combinations, 6 groups of pesticides could be identified which were substantially similar to groups of pesticides we had formed based on chemical structure and overall genetic activity (Waters et al., 1982). The matches showed excellent qualitative and, in most cases, excellent quantitative agreement. It seems reasonable, therefore, that specific patterns of genetic test results present in the overall profiles are related directly to chemical structure.

Thus, we have been able to demonstrate that (1) pesticides with similar structures produce similar profiles of genetic activity, (2) certain test systems may be more appropriate for some classes of pesticides, and (3) certain groups of compounds may be recognized by a well defined series of concordant test results. Structure–activity correlations based on a comparative evaluation of genetic activity profiles representing a variety of test systems should provide useful information relative to possible mechanisms of genetic toxicity. As more information is gained, comparison of preliminary test results for new chemicals with existing data for known genotoxicants should aid in the evaluation of potential health hazards.

References

Casida, J.E., E.C. Kimmel, M. Lay, H. Ohkawa, J.E. Rodebush, R.A. Gray, C.K. Tseng and H. Tilles (1975) Environ. Qual. Safety, 3: Suppl., 675.

Chan, P.K., G.P. O'Hara and A.W. Hayes (1982) Principles and methods for acute and subchronic toxicity, in: A.W. Hayes (Ed.), Principles and Methods of Toxicology, Raven, New York.

Eto, M. (1974) Organophosphorus Pesticides: Organic and Biological Chemistry, CRC Press, Cleveland, OH, 387 pp.

Eto, M. and H. Ohkawa (1970) Alkylation reaction of organophosphorus pesticides: its chemical and biochemical significances, in: R.D. O'Brien and I. Yamamoto (Eds.), Biochemical Toxicology of Insecticides, Academic Press, New York, p. 93.

Garrett, N.E., H.F. Stack, M.R. Gross and M.D. Waters (1984) An analysis of the spectra of genetic activity produced by known or suspected human carcinogens, Mutation Res., 134, 89–111.

Griesemer, R.A., and C. Cueto Jr. (1981) Toward a classification scheme for degrees of experimental evidence for the carcinogenicity of chemicals for animals, in: R. Montesano, H. Bartsch and L. Tomatis (Eds.), Molecular and Cellular Aspects of Carcinogen Screening Tests, Vol. 27, IARC Scientific Publications, IARC, Lyon, France, pp. 259–281.

IARC (1982) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Suppl. 4, Chemicals, Industrial Processes and Industries Associated with Cancer in Humans, IARC Monographs, Vols. 1–29, 292pp.

IARC (1985) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 36, Some Allyl and Allylic Compounds, Aldehydes, Epoxides and Peroxides, Appendix: Activity Profiles for Short-Term Tests, Lyon, France, pp. 325–346.

Innes, J.R.M., B.M. Ulland, M.G. Valerio, L. Petrucelli, L. Fishbein, E.R. Hart, A.J. Pallotta, R.R. Bates, H.L. Falk, J.J. Gart, M. Klein, I. Mitchel and J. Peters (1969) Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note, J. Natl. Cancer Inst., 42, 1101.

Kochetkov, N.K. and E.I. Budovskii (1972) Organic Chemistry of Nucleic Acids, Part B, Chapter 5, Plenum, New York, pp. 269–348.

Kohn, G.K. (1977) The sulfenimide fungicides, in: J.F. Plimmer (Ed.), Pesticides Chemistry in 20th Century, ACS Symp. Ser. 37, Washington, DC, pp. 153–169.

Moore, M.M., D. Clive, J.C. Hozier, B.E. Howard, A.G. Batson, N.T. Turner and J. Saywer (1985) Analysis of trifluorothymidine-resistant (TFT^r) mutants of L5178Y/TK$^{+/-}$ mouse lymphoma cells, Mutation Res., 151, 161–174.

NAS (1985) Evaluation of Cyclamate for Carcinogenicity, National Academy Press, Washington, DC, pp. 67–70.

Schuphan, I., J.D. Rosen and J.E. Casida (1979) Science, 205, 1013.

Schuphan, I., Y. Segall, J.D. Rosen and J.E. Casida (1981) Toxicological significance of oxidation and rearrangement reactions of *S*-chloroallyl thio- and dithio-carbamate herbicides, in: J.D. Rosen, P.S. Magee and J.E. Casida (Eds.), Sulfur in Pesticide Action and Metabolism, ACS Symp. 158, Washington, D.C. pp. 65–82.

Vainio, H., M.D. Waters and H. Norppa (1985) Mutagenicity of selected organic solvents, Scand. J. Work Environ. Health, 11 (Suppl. 1), 75–82.

Waters, M.D. (1979) The Gene-Tox Program, in: Banbury Report 2, A.W. Hsie, J.P. O'Neill and V.K. McElheny (Eds.), Cold Springer Harbor Laboratory Press, Cold Spring Harbor, New York pp. 449–467.

Waters, M.D., H.F. Stack, K.H. Mavournin and V.L. Dellarco (1985) Special Committee Report, Part II: Quantitative evaluation of chemicals that induce aneuploidy using the genetic activity profile method, in: V.L. Dellarco, P.E. Voytek and A. Hollaender (Eds.), Aneuploidy: Etiology and Mechanisms, Plenum, New York, pp. 455–490.

Waters, M.D., H.F. Stack, K.H. Mavournin and V.L. Dellarco (1986) Genetic activity profiles of chemicals selected from the aneuploidy data base, Mutation Res., 167, 171–188.

Waters, M.D., S.S. Sandhu, V.F. Simmon, K.E. Mortelmans, A.D. Mitchell, T.A. Jorgenson, D.C.L. Jones, R. Valencia and N.E. Garrett (1982) Study of pesticide genotoxicity, in: R. Fleck and A. Hollaender (Eds.), Genetic Toxicology — An Agricultural Perspective, Plenum, New York, pp. 275–326.

Zetterberg, G. (1977) Genetic effects of phenoxy acids on microorganisms, Ecol. Bull. (Stockholm), 27, 193.